

ORIGINAL ARTICLE

DIRECT COOMB'S TEST IN HYPERBILIRUBINEMIA OF THE NEWBORN

Pradeep Kumar B, Shashidhar A, Sitalakshmi D*, Suman Rao PN

Abstract

Aim: To evaluate the association of hyperbilirubinemia and Direct Coomb's Test (DCT) in ABO/ Rh incompatibility setting.

Methods: A comparative study of DCT positive and DCT negative newborns by retrospective chart review was done in a tertiary care neonatal center of Southern India between January 2015 to December 2016. Bilirubin levels, phototherapy duration, intravenous immunoglobulin (IVIG) use, exchange transfusion rates were compared between the 2 groups.

Results: Out of the 140 babies reviewed, 36 (25.7%) newborns were DCT positive and 104 (74.3%) were DCT negative. Bilirubin centile >95th centile was seen in 16 (44.5%) in DCT positive and 18 (17.5%) in DCT negative group ($p=0.009$). Babies in DCT positive group had higher duration of phototherapy (62.5 ± 26.7 hours) as compared to the DCT negative group (46.1 ± 23.5 hours) ($p=0.002$), longer hospital stay (5.9 ± 3.5 days versus 4.8 ± 1.8 days) ($p=0.01$), higher reticulocyte count ($9.4\pm 4.9\%$ vs $5.7\pm 2.5\%$) ($p=0.001$). Four babies in DCT positive group required exchange transfusion whereas none in the DCT negative group needed exchange transfusion ($p=0.004$), 10 (27.8%) needed IVIG in DCT positive group as compared to 3 (2.9%) in DCT negative group ($p=0.0001$) and neonatal intensive care unit (NICU) admissions were needed in 15 (41.7%) in DCT positive as compared to 8 (7.6%) in DCT negative group ($p=0.0001$).

Conclusion: A positive DCT at birth is associated with higher bilirubin levels and need for multiple interventions for jaundice management.

Keywords : Direct Coombs Test; Hyperbilirubinemia, Neonatal; Phototherapy; Blood Group Incompatibility

Introduction

Direct Coomb's test (DCT) is a test for detecting antibodies present on the red blood cells (RBCs) and is used predominantly in the diagnosis of hemolytic disease of the newborn (HDN). A positive DCT in a newborn suggests the transplacental transfer of IgG antibodies present in maternal serum and directed against antigens on fetal and neonatal RBCs. These antibodies can result in immune mediated hemolysis and shortened RBC life span, leading to varying severity of hyperbilirubinemia and anemia. The factors that lead to a positive DCT in neonates are mainly the ABO and Rh incompatibility between the newborn and the mother, and rarely maternal autoimmune hemolytic disease. Blood group incompatibility with a positive DCT is considered a major risk factor for the development of severe hyperbilirubinemia and neurotoxicity. (1) However, the utility and effectiveness of this test has been questioned in literature. (2,3) We did this study to determine the association of hyperbilirubinemia and DCT in ABO/ Rh incompatibility setting.

Methods & Materials

This comparative study was conducted in a tertiary care neonatal intensive care unit (NICU) in Southern

India between January 2015 till December 2016. The inclusion criteria were newborn babies in whom DCT was done during the period of the study in ABO or Rh incompatibility setting. They were divided on the basis of test result into DCT positive and DCT negative group. We excluded babies whose mothers were diagnosed with autoimmune hemolytic anemia [e.g. systemic lupus erythematosus (SLE), Evans syndrome] and where a valid test result could not be found in the hospital records. The institutional ethics committee approved the study and granted a waiver of consent. Cord blood/initial blood samples of babies born to mothers with O blood group/ Rh negative blood group were routinely sent for DCT as per hospital protocol. A confirmatory DCT was done from the baby's serum whenever cord blood results were positive/equivocal which was reported by a senior pathologist. For the purpose of the study only those babies with confirmed positive test in the serum were considered positive irrespective of cord blood results. From the Blood Bank records, the following data was collected: DCT results, infant/maternal ABO and Rhesus D group. A retrospective review of the charts of infants with a positive/negative DCT was performed. The following characteristics were recorded: gender, gestational age, mode of delivery, maternal medical history, peak serum bilirubin, treatment duration with phototherapy, exchange transfusion requirement and length of hospital stay. The groups (DCT Positive & DCT negative) were compared for the outcomes and the result of the DCT. The hospital policy followed during the period of the study was that a serum bilirubin was sent for a baby at 48 hours with risk factors like prematurity, suspected hemolysis, gestational diabetes etc. or clinically when the icterus was upto or below the level of abdomen or when the DCT result was positive. Bilirubin was monitored every 8-12 hours in babies with hemolysis and every 24 hours in babies requiring phototherapy. In case of isoimmunisation/hemolysis, phototherapy was started empirically and in all other cases as per American Academy of Pediatrics (AAP) 2004 guidelines. (1) Phototherapy was stopped when the bilirubin value was <13 mg/dl. Exchange transfusion was considered when there was no response to phototherapy as per AAP recommendations and intravenous immunoglobulin (IVIG) was given when the bilirubin levels were close to the exchange range. The centiles for serum bilirubin were calculated using the hour specific nomogram by Bhutani et al (4). The risk zone on the nomogram was characterized as follows: Zone a - low risk <40th percentile; Zone b - low to intermediate risk: 40-75th percentile; Zone c - high to intermediate risk: 76-95th percentile; and zone d, high risk > 95th percentile.

Sample size: The study by Valsami et al (5) reported that 42% of positive DCT cases would undergo phototherapy. Assuming that about 10% of DCT negative subjects would undergo phototherapy, the sample size required for examining the difference in hyperbilirubinemia and phototherapy between DCT positive and negative subjects with 80% power and

Table 1: Baseline characteristics of the two groups

Base line characteristics	DCT Positive (n=36)	DCT Negative (n=104)
Male	14 (42%)	52 (50%)
Female	21 (58%)	52 (50%)
Inborn	31 (86%)	104 (100%)
Term	35 (97%)	98 (94%)
Primigravida	17 (47%)	52 (50%)
Previous Sibling death/IUD	3 (3%)	9 (9%)
Anti-D received	2	8
Mother's ICT positive	1	0
Gestational Diabetes	3 (8%)	15 (14%)
Maternal Blood group O/ Baby's blood group A	16 (45%)	35 (34%)
Maternal Blood group O/ Baby's blood group B	18 (50%)	50 (48%)
Maternal blood group Rh-/ Baby's Blood group Rh+	2 (5%)	19 (18%)

Note: IUD – intrauterine death, ICT – Indirect Coomb's test

Table 2a: Outcome parameters in both the groups

Variable	DCT Positive (n=36)	DCT Negative (n=104)	P value
Peak Bilirubin (mg/dl)	13.5 ± 5.1	12 ± 3.4	0.06
Peak of jaundice (hours of life)	61 ± 38.9	64.3 ± 24.2	0.5
Lowest PCV (%)	43.2 ± 8.6	47.5 ± 7	0.14
Duration of Phototherapy (hours)	62.5 ± 26.7	46.1 ± 23.5	0.002
Reticulocyte Count (%)	9.4 ± 4.9	5.7 ± 2.5	0.001
Mean Hospital stay (days)	5.9 ± 3.5	4.8 ± 1.8	0.01

Note: PCV – packed cell volume,

Table 2b: Outcome parameters in both the groups

Variable	DCT Positive (n=36)	DCT Negative (n=104)	P value
Exchange Transfusion	4 (11.1%)	0	0.004
BIND	1 (2.8%)	0	0.25
IVIG requirement	10 (27.8%)	3 (2.9%)	0.0001
NICU admission for intensive phototherapy	15 (41.7%)	8 (7.6%)	0.0001

Note: BIND – bilirubin neurological dysfunction, IVIG- intravenous immunoglobulin

5% level of significance was 28 in each group. The power of the study was increased by recruiting about 3 times this number in the control group.

Statistical analysis was performed using the t test for continuous data, and Fisher's exact test or the chi-square test for categorical data as appropriate. Data were analyzed with the SPSS.20 software and a p value of 0.05 or less was considered statistically significant.

Results

We analyzed 140 babies during the study period of 2 years. Thirty- six (25.7%) newborns were in DCT positive group and 104 (74.3%) in DCT negative group. Baseline characteristics of both the groups are depicted in Table 1. The baseline variables were

comparable in each group. Bilirubin centile <40th was seen in 8 (22.2%) of the DCT positive and 24 (23%) of the DCT negative group, centile between 41-75th was seen in 7 (19.4%) in DCT positive and 34 (32.6%) in DCT negative, centile between 76-95th was seen in 5 (13.9%) in DCT positive and 28 (26.9%) in DCT negative and >95th centile was seen in 16 (44.5%) in DCT positive and 18 (17.5%) in DCT negative group (p=0.009). Other outcome parameters in both the groups are depicted in Table 2a and 2b.

Discussion

In our study, a positive DCT was associated with a higher risk of developing significant jaundice (>95th centile for the age) which in turn resulted in greater need for interventions (phototherapy, NICU admissions,

exchange transfusion and IVIG). These results are similar to the studies done earlier. (6-8). This study reemphasizes the need to screen babies at risk for blood group incompatibility (where mother's blood group is O, or Rh negative and baby's blood group is A or B or Rh positive respectively) with a DCT soon after birth. These babies need to be monitored aggressively for the development of severe hyperbilirubinemia and need for adjunctive treatments.

As a screening test, a positive DCT does not either rule in or exclude the probability of hemolysis (though we found a significantly higher reticulocyte count) and only indicates antibody-mediated red cell binding with false positive and false positive reports. Though it has been identified as having a poor positive predictive value (PPV) in general population for identifying newborns at risk of clinically significant hyperbilirubinemia (9), in selective high risk population it may be still beneficial. (10) The anti-globulin or Coomb's test needs to be included in the decision making algorithms to improve prediction of significant neonatal hyperbilirubinemia in order to optimize screening protocols and safe discharge of neonates.

The limitations of this study were the retrospective methodology, and a policy of starting empirical phototherapy for all DCT positive newborns while awaiting lab results of bilirubin. This could have increased the number of babies receiving phototherapy in the DCT positive group, and prevented the acute rise of bilirubin which is probably the reason why the peak bilirubin levels were not different between the groups. However, this would not have influenced any of the other outcomes. We did not intend to study the utility of DCT in minor blood group incompatibility or its cost effectiveness or the incidence & predictive value of DCT. We recommend a multicentric prospective study to further answer these pertinent questions.

In conclusion, a positive DCT at birth is associated with higher incidence of greater hyperbilirubinemia and a greater need for multiple interventions.

Funding : None

Conflict of Interest : None

References :

1. Subcommittee-on-Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* 2004;114:297e316.
2. Han P, Kiruba R, Ong R, Joseph R, Tan KL, Wong HB. Haematolytic disease due to ABO incompatibility: Incidence and value of screening in an Asian population. *Aust Paediatr. J.* 1988; 24 (1): 35-8. 10
3. Meberg A, Johansen KB. Screening for neonatal hyperbilirubinemia and ABO alloimmunization at the time of testing for phenylketonuria and congenital hypothyreosis. *Acta Paediatr.* 1998; 87 (12): 1269-74
4. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischage hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics* 1999;103:6e14
5. Valsami S, Politou M, Boutsikou T, Briana D, Papatesta M, Malamitsi-Puchner A. Importance of Direct Antiglobulin Test (DAT) in Cord Blood: Causes of DAT (+) in a Cohort Study. *Pediatr Neonatol* 2015; 56:256.
6. Sarici SU, Yurdakok M, Serdar MA, Oran O, Erdem G, Tekinalp G, et al. An early (sixth-hour) serum bilirubin measurement is useful in predicting the development of significant hyperbilirubinemia and severe ABO hemolytic disease in a selective high-risk population of newborns with ABO incompatibility. *Pediatrics* 2002; 109 (4): e53
7. Chen JY, Ling UP. Prediction of the development of neonatal hyperbilirubinemia in ABO incompatibility. *Zhonghua Yi Xue Za Zhi (Taipei)* 1994; 53 (1): 13-1
8. Madlon-Kay DJ. The clinical significance of ABO blood group incompatibility. *Arch Fam Med.* 1993; 2 (3): 285-7
9. Keir A, Agpalo M, Lieberman L, Callum J. How to use: the direct antiglobulin test in newborns. *Arch Dis Child - Educ Pract Ed [Internet].* 2015 Aug [cited 2017 Sep 9]; 100(4):198-203.
10. Maisels MJ, Watchko JF. Routine blood typing and DAT in infants of group O mothers. *J Perinatol* 2013;33:579.

From: Departments of Neonatology and *Transfusion Medicine, St. John's Medical College, Bangalore, India.

Address for Correspondence:

Dr. Shashidhar A, Associate professor, Department of Neonatology, St John's Medical College Bangalore.

Email : shashiishere@gmail.com

DOI: 1510.7199/ped.oncall.2018.15

